CONSTITUENTS OF THE UNSAPONIFIABLE FRACTION IN THE OIL OF

Mangifera indica and Oreodoxa regia

By

Seham Fiad, Ph.D., **F.M. Ali**, M. Sc.

Department of Fats

and Oils, National Research Centre, Dokki, Cairo.

and

Mahassen H. Abbasy, Ph.D.

University College for Girls, Ain Shams University.

INTRODUCTION

The exact composition the unsaponifiable fraction of mango oil is not yet completely known. A sitosterol was identified by Bajpal and Mukherjee (1967), but no details about its nature were given.

The work done by Corsano and Piacatelli (1965) and Corsano and Mincione (1965, 1967) dealt with the isolation, identification and finally the structure of many of the naturally occuring terpenes especially triterpenes, in the resin present in mango oil. Two fractions were identified in mango resin : an acidic fraction and a neutral fraction. Chromatography of the acidic fraction of the resin on silica gel gave mangeferolic acid and about 8% hydromangerolic acid (Corsano and piacatelli, 1965). Further work on this fraction (Corsano and Mincione 1965, 1967) revealed the additional presence of isomangeferolic acid and ambonic acid. Corsano and Mincione (1968) studied the strucure of mangeferolic, isomangeferolic and hydroxymangeferolic acids.

Earlier Corsano and Mincione (1965) had chromatographed the neutral fraction of the resin on silica gel and eluted with different concentrations of hexane : ether. Three compounds were obtained from the column, (I) oleanolic aldehyde, m.p. 168 - 172°C $[\alpha]_D$ 72° ; (II) a substance with m.p. 166 - 170°C, $[\alpha]_D$ 41° ; (III) a substance with m.p. 154 - 157°C, $[\alpha]_D$ 54°. The strucures of these

compounds were studied on the basis of N.M.R. spectra. Moreover, the neutral fraction was found to contain cycloartenol acetate, m.p. 124 - 125°C ; B-amyrin acetate 236 - 240°C and lupeol acetate.

The knowledge of the unsaponifiable fraction of the oil from the kernels of Oreodoxa regia is limited. Jasperson and Jones (1947) had found in the unsaponifiable fraction of palm oil, that the predominant type of hydrocarbon is a terpenoid. Mellier (1951) obtained a mixture of sterols from the soap of palm oil by salt precipitation, the purified sterol mixture contained mainly a sitosterol with a trace of ergosterol. Argound (1954) studied the chromatograms of the unsaponifiable matter of palm oil and recorded the presence of phytoene, phytoflavone, d-carotene and tetrahydrocarbon lycopene, on using ultraviolet spectroscopy. Fedeli et al. (1966) studied the unsaponifiable fraction of the oils extracted from various plants by means of gas liquid chromatography in order to separate the triterpene alcohols and sterols. In palm and palm/kernel oils, two tritepene alcohols, cycloartenol and 24-methylene cycloartenol were found. Karleskind et al. (1966) found B-sitosterol, stigmasterol and campesterol, moreover a fourth unidentified sterol was found to be present in palm and palm kernel oils.

From the literature it is clear that the knowledge of the exact composition of the unsaponifiable fraction of mango and palm oils is still limited. In the present study an attempt was made to throw some more light on the subject.

EXPERIMENTAL

The oil from Kernels of the mango makes up 10% of the Kernel substance. Saponification of the oil was done by alcoholic potash ; the unsaponifiable fraction of the oil was 2.3%. The resolution of this fraction was effected by chromatography on alumina, column. The early fraction of the chromatogram when eluted with hexane afforded a waxy product constituting 6.9% of the unsaponifiable matter. This substance is evidently a saturated hydrocarbon which was not further investigated.

Further elution with benzene : hexane (75 : 25) and pure benzene yielded a solid substance 1, giving the colour reaction of terpenes. Consequent elution with benzene : methyl alcohol (95 : 5) gave another solid substance 11, showing the colour reactions of sterols. These two fractions I and II were further investigated.

Chromatography of fraction I, using silica gel G thinlayers and using a developing system of (100 : 15) cyclohexane : ethyl acetate

(Osman 1965), revealed the presence of two terpenes. This was further proved by the following tests : treatment with antimony trichloride reagent resulted in the appearance of two separate pink spots, Lieberman Burchardt's test caused red coloration wheras concentrated sulfuric acid gave lemon greenish fluorescence.

The two terpenes were separated by means of the preparative thinlayer technique, the major one had R_f 0.40 and the minor one had R 0.38. The major terpene had a melting point 196 - 197°C ; $[\alpha]_D$ 87° ; the melting point was not depressed by authentic B-amyrin, from which it is moreover not separable when subjected to thinlayer chromatography. The minor terpene with R_f 0.38 had a melting point 215 - 216°C ; $[\alpha]_D$ 26.4° ; the melting point was not depressed by addition of authentic lupeol and also not separable from it by thinlayer technique.

The identification of these two terpenes as B-amyrin and lupeol was further confirmed by preparation of their acetates.

Crystalisation of fraction II from chloroform : methyl alcohol (50 : 50) gave a white solid material responding to the colour reactions of sterols. It gave a green Lieberman-Burchardt's test and a yellow colour with tetranitromethane reagent which latter indicates its unsaturation character. The ultraviolet spectrum (in ethanol) exhibited a maximal absorption at 205 mu (t = 3200), this was not high enough to account more than one ethylenic linkage. The material, with m.p. 135 - 136°C ; $[\alpha]_D$ - 37° ; was evidently uniform as indicated on silica gel G chromatoplates. The negative optical rotation was suggestive of a location of the double bond in the 5 : 6 position. This was also supported by the examination of the infrared absorption spectra of the sterol, which contained (besides the hydroxyl absorption near 3300 cm-1) two peaks at 840 and 803 cm-1 attributable to a trisubstituted double linkage (Fig. 1). The digitonin test for this sterol was positive i.e. giving an insoluble white digitonide, thus indicating a free 3-B hydroxy group. All these properties in addition to their physical constants are strong evidence that the sterol is identical with B-sitosterol (Fig. 2). This was confirmed by the method of Osman (1965), when the sterol spot R_f 0.32 remained unchanged after mixing with authentic B-sitosterol.

For further establishment of the identity of the sterol, the acetyl as well as the benzoyl derivatives were prepared. The acetate derivative (m.p. 123 - 124°C, $[\alpha]_D$ - 41.1°) as well as the benzoate derivative (m.p. 145 - 146°C ; $[\alpha]_D$ - 14.5°) were prepared and were found to be identical in all respects with authentic

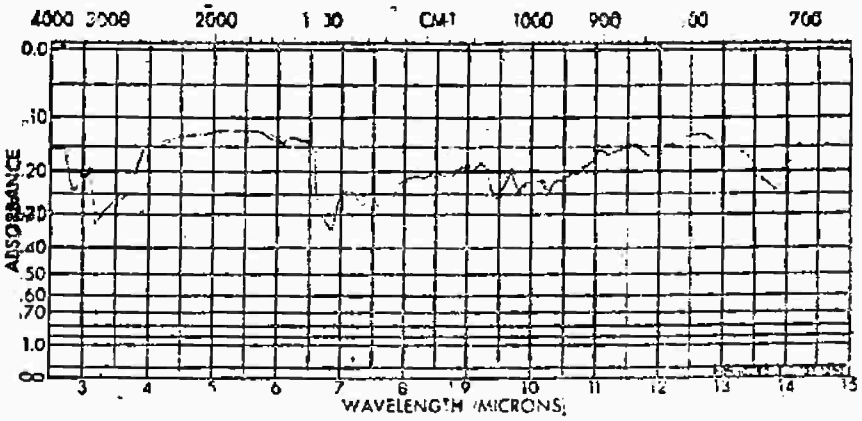
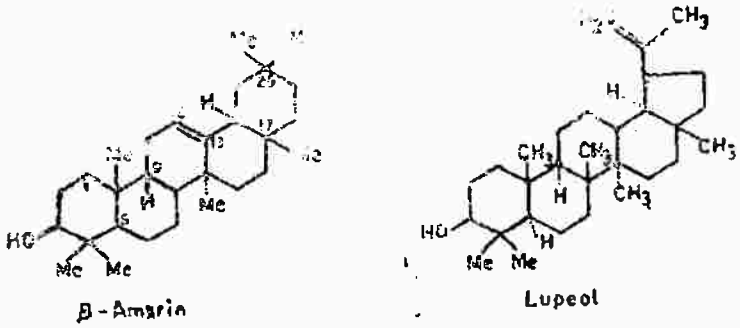
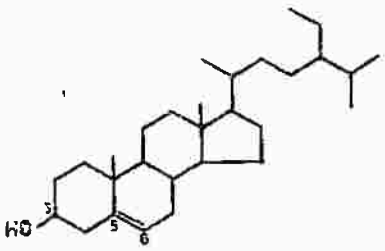


Fig. (1)



β-Amyrin Lupeol

Fraction (I)



β-Sitosterol

Fraction (II)

Fig. (2)

sitosterol acetate and benzoate. The $[\alpha]_D$ measurements were done in Chloroform in all experiments. These results confirm and extend the data of Bajpal and Mukherjee (1967).

The oil of *Oreodoxa regia* which forms 32% of the whole fruits, was saponified and 7.9% unsaponifiable matter was obtained. The resolution of its fractions was effected by chromatography on alumina column. The early fraction eluted with n-hexane was a waxy hydrocarbon product constituting 9.0% of the unsaponifiable matter.

After this hydrocarbon, a resin was removed by hexane : benzene (1 : 3) and with pure benzene. This resin (30%) gave a positive Lieberman-Burchardt's test (red colour) which indicates the presence of terpene matter. When this terpene was examined by the thin-layer technique, it gave a mixture of three unidentified components. The R_f values of these components were 0.32, 0.36 and 0.38 ; this mixture could not be crystallised or even purified by preparative thin-layer technique.

When the alumina column was eluted with 1-5% methyl alcohol in benzene, the same above mentioned mixture of the three terpenes was obtained as shown from results obtained by their chromatography on thinlayer (Fig. 3). Moreover a fourth major component was obtained, the R_f of this fourth component was 0.25 which suggested its identity as a trerol.

Preparative thinlayer chromatography was carried out on the above mixture to obtain the fourth polar component having R_f 0.25, its uniformity was checked by chromatography. The isolated component responded to Lieberman-Burchardt's test for sterols giving green colour. The unsaturation of the isolated sterol was evident from the yellow coloration it gave with tetranitromethane reagent.

This component could not be crystallised from different solvents, not even could its acetate and benzoate derivatives be obtained, a fact which indicated that this compenent was not a simple sterol but a mixture of sterols. Accordingly, the benzoate of this mixture was analysed chromatographically by means of a wedge shaped thinlayer using silver nitrate. The spots were detected by antimony trichloride reagent. It was evident from the experiments on adding authentic substances to the sterol mixture, that this mixture contained three components corresponding to B-sitosterol, stigmasterol and campesterol.

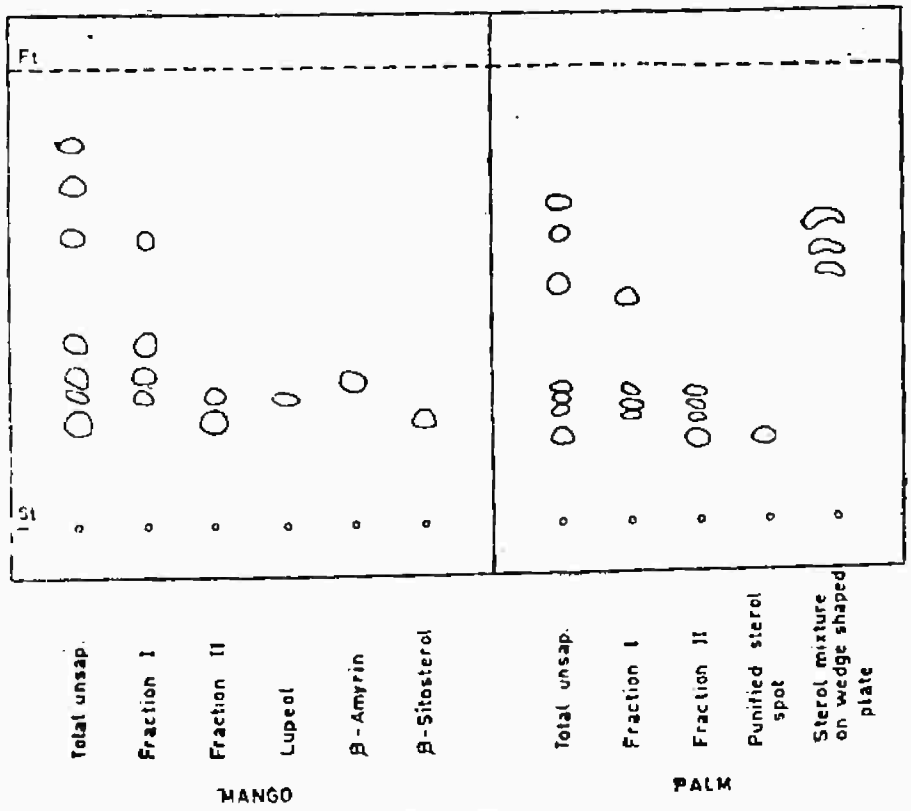


Fig. (3)

SUMMARY

amyrin, lupeol and B-sitosterol have been isolated from the unsaponifiable matter obtained from the oil of the kernels of *Mangifera indica*, in a yield of about 59% for the terpenes and 23% for the sterol.

Oreodoxa regia oil contains a mixture of three triterpenes which could however not be identified further. Morover, B-sitosterol, stigmasterol and campesterol were proved to be the components of the sterol mixture found in palm oil.

REFERENCES

Argound, S., Oleagineux 9, 717 (1954).

Bajpal, K.S. and Mukherjee, S., Labdev. Kapur, India 5, 152 (1967).

Corsano, S. and Mincione, Ric. Sci. 37,370 (1965).

Corsano, S. and Mincione, É., Ann. Chim. 57, 522 (1967).

Corsano, S. and Mincione, E., Ric. Sci. 38, 537 (1968).

Corsano, Sa and Piacatelli, G., Univ. Rome, Sci. Rend, Sez. A 8, 484 (1965).

Fedeli, F., Lanzani, A., Capella, p. and Jacini, G., J.A.O.C.S. 43, 245 (1966).

Jasperson, H. and Jones, R., J. Soc. Chem. Ind. 66,13 (1947).

Karleskind, A., Audiou, F. and Wolff, J.P., Rev. France Corps. Gras. 13, 165 (1966).

Mellier, M.T., Oleagineux 6,2 (1951).

Osman, F., ph. D. Thesis Cairo University (1965).